Published in final edited form as:

Science. 2016 January 8; 351(6269): 180–186. doi:10.1126/science.aad3460.

The structure of the β -barrel assembly machinery complex

Jeremy Bakelar¹, Susan K. Buchanan², and Nicholas Noinaj^{1,*}

¹Markey Center for Structural Biology, Department of Biological Sciences, Purdue University, West Lafayette, Indiana, 47907

²National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, Maryland, 20892

Abstract

β -barrel outer membrane proteins (OMPs) are found within the outer membranes (OM) of Gram-negative bacteria and are essential for nutrient import, signaling, and adhesion. While the exact mechanism is unknown, a 200 kDa five component complex called the β -barrel assembly machinery (BAM) complex has been implicated in the biogenesis of OMPs. Here, we report the structure of the BAM complex from *E. coli*, revealing that binding of the accessory proteins BamCDE modulates the conformation of BamA, the central component of the complex, which may regulate the function of the BAM complex. The periplasmic domain of BamA was found in a closed state that prevents access to the barrel lumen from the periplasm, indicating substrate OMPs likely do not enter the barrel during biogenesis. Further, the first eight strands of the β -barrel domain undergo an unprecedented conformational shift leading to opening of the exit pore and rearrangement at the lateral gate.

Gram-negative bacteria contain both an inner membrane (IM) and an outer membrane (OM) which serve important roles in nutrient import, cell signaling, waste export, and protection. The IM exclusively contains integral membrane proteins that have an α -helical fold consisting of one or more α -helices which anchor the protein into the membrane. The OM, however, contains almost exclusively integral membrane proteins that have a β -barrel fold consisting of 8–26 antiparallel β -strands which anchor the protein in the OM. Like α -helical membrane proteins, β -barrel outer membrane proteins (OMPs) serve many functions and therefore have diverse structural and biochemical properties. In pathogenic strains of bacteria, some OMPs can also serve as virulence factors that mediate infection. In nature, OMPs are only found in the OMs of Gram-negative bacteria, mitochondria, and chloroplasts (1, 2).

*Correspondence to: ; Email: nnoinaj@purdue.edu

Supplementary Materials:

Materials and Methods

Figures S1–S6

Tables S1–S6

Movies S1–S2

References (35–40)

Despite their essential roles, the exact mechanism explaining how OMPs are folded and inserted into the OM remains unknown. In the last decade, studies have identified a general pathway and conserved machinery that is responsible for the biogenesis of OMPs (3–5). A majority of these advances have been made working with Gram-negative bacteria (3, 6, 7). Here, the nascent OMPs are first synthesized in the cytoplasm with an N-terminal leader sequence which directs them to the Sec translocon for transport across the IM, into the periplasm (Figure 1A). Chaperones such as SurA and Skp then further escort the nascent OMPs to a multicomponent complex called the β -barrel assembly machinery (BAM) complex, which has been shown to be responsible for folding and inserting OMPs into the OM (4, 8). In *E. coli*, the BAM complex consists of five components called BamA, BamB, BamC, BamD, and BamE. BamA, a 16-stranded OMP itself, is the central and essential component of the complex and is conserved both in mitochondria and chloroplasts. BamB, BamC, BamD, and BamE are all lipoproteins which are anchored to the OM via lipidation of the N-terminal cysteine residue. BamA and BamD are essential for viability; however, all components are required for efficient OMP folding/insertion (9–11). Studies have shown that both BamB and BamD interact directly with BamA via non-overlapping binding sites while BamC and BamE interact directly with BamD to stabilize the complex (9, 12).

Structures of all the Bam components have now been reported including partial complexes of BamAB and BamCD (7, 13–23). The full-length structure of BamA from *Neisseria gonorrhoeae* revealed a large periplasmic domain consisting of five polypeptide transport associated (POTRA) domains and a C-terminal 16-stranded β -barrel domain. Subsequent studies showed that lateral opening of the barrel domain was required for function in BamA, strengthening an existing hypothesis that the barrel domain must open laterally in the membrane to allow insertion of the substrate OMPs into the OM (16, 18, 24). It has been proposed that BamB might serve as a scaffold, assisting in the handoff of nascent OMPs by SurA/Skp to BamA, while BamC, BamD, and BamE may serve support roles in regulating the function of BamA (14, 17, 25). The structures have offered clues to how each component may function within the complex; however, the lack of structural information regarding the fully assembled complex has hindered progress towards exploring the mechanism further. To address this, we report here the structure of the BAM complex from *E. coli*. We used X-ray crystallography to solve the crystal structure of the BamACDE subcomplex to 3.4 Å resolution and utilized the previously reported partial structure of BamAB to form a model for the fully assembled BAM complex. Our structure shows the periplasmic domain of BamA in a closed state that prevents access to the barrel lumen from the periplasm. Furthermore, binding of BamCDE to BamA causes an unprecedented conformational twisting of strands β 1– β 8, leading to opening of the top of the barrel domain along the exit pore and structural rearrangement of the lateral opening site. These structural changes suggest that the role of BamCDE may be to modulate the conformational states of BamA, thereby serving as a regulatory step in the function of the BAM complex.

The BAM complex from *E. coli* was expressed from a single plasmid and purified as previously described with some modifications (11). SDS-PAGE analysis verified the presence of the full complex which produced a monodisperse peak by size exclusion chromatography (Figure S1). We were able to crystallize the complex by detergent screening using C₈E₄ and collected data at the SER-CAT ID22 beamline at the Advanced Photon

Source. We solved the crystal structure of the complex to 3.4 Å resolution by molecular replacement (Figure S2 and Table S1). However, it was clear that BamB was not present, possibly due to proteolytic degradation during incubation. This was verified by SDS-PAGE analysis of crystals and of the original protein sample, both of which lacked BamB after extended incubation/storage (Figure S1). Our crystal structure contains full length BamA, BamD, and BamE, but only the N-terminal flexible domain and the first globular helix-grip domain of BamC with the second helix-grip domain presumably being disordered. To model of the fully assembled BAM complex, we utilized the previously reported structure of the partial BamAB complex (PDB ID 4PK1) (14) to dock BamB into our crystal structure by aligning along POTRA3 of BamA (Figures 1B, C, Movie S1, and Model S1). The overall dimensions of the BAM complex are ~115 Å in width and height. BamD interacts with POTRA5 and POTRA2 of BamA while BamE interacts with both BamD and with POTRA5. BamC was found to interact with BamD in the same manner as has been previously reported (13). For all accessory lipoproteins, the N-terminal residues are positioned in close proximity to where the OM would sit; however, no lipid anchors were observable in our crystal structure.

While the structure of the BamCD complex had been reported previously (13), no structure of the BamCDE complex has been described. In our structure, BamC interacts with BamD primarily along its elongated intrinsically disordered, N-terminal domain with a buried surface area of ~2,300 Å², while BamE interacts along the C-terminal end of BamD with a buried surface area of ~800 Å² (Figure 2A and Tables S2, S3). BamCD in our structure aligned very well with the previously reported complex (PDB ID 3TGO), having an RMSD of 1.25 Å across both chains (Figure 2B). While BamC and BamE interact with BamD via non-overlapping binding sites, they do make contact with one another through a buried surface area of ~140 Å² along residues 67–69 of BamE and residues 55–59 of BamC. Residues 215–344 of BamC consisting of a linker and the second helix-grip domain were not observed in our crystal structure and presumed to be disordered. This region of the structure sits adjacent to a very large open cavity in the crystal lattice which does not provide sufficient contacts for stabilizing this flexible region of the structure. Previous studies have shown that the two helix-grip domains of BamC are surface exposed (26), suggesting that one or both may interact directly with the surface of BamA. In our crystal structure, no direct interaction was observed indicating that if the two helix-grip domains of BamC do indeed interact with BamA, a membrane bilayer and/or substrate may be required to release BamC from BamD so it can be presented on the surface.

The structure of BamD consists of five tetratricopeptide-repeat (TPR) motifs (15, 21, 27), with TPR4 and 5 forming the binding site for BamE, which was found oriented perpendicular to the membrane (Figure 2A). The binding interface agrees well with previously published work, where BamD was found to bind BamE along the interface containing residues R29, I32, R78, F68, N71, T72, and T92 (23). In our structure, the extensive binding interface has numerous hydrogen bonds using both side chain and backbone atoms, and a salt bridge between D66 of BamE with K233 of BamD (Figures 2C, D). The binding is further strengthened by interactions of M64 and F68 of BamE into hydrophobic pockets in BamD. The minimal interaction between BamC and BamE is

mediated primarily through hydrophobic interactions; the loop of BamC helps to form the large hydrophobic pocket where F68 of BamE binds BamD (Table S4).

In agreement with previous studies (28), BamD was found to bind BamA along POTRA5 almost exclusively via TPR3 and 4, sitting parallel to the membrane with a buried surface area of $\sim 1,100 \text{ \AA}^2$ (Figures 3A, B and Table S5). Interestingly, TPR1 and 2 of BamD also interact with POTRA2 of BamA through a buried surface area of $\sim 115 \text{ \AA}^2$. Whether this minimal interaction is real or an artifact of crystallization remains to be determined. The extensive binding interface between BamD and POTRA5 of BamA is mediated by many hydrogen bonds using both side chain and backbone atoms and contains three salt bridges between residues H139, R197, and R188 of BamD and residues D358, E373, and D481 of BamA, respectively (Figures 3B, C). The salt bridge formed by R197 of BamD and E373 of BamA is central to this interaction and these residues have previously been determined to play an essential role in binding via a putative salt bridge interaction, as our structure confirms (29, 30). The extended loop of BamD TPR3 (residues 123–130) also interacts with periplasmic loop 1 of BamA along residues 449–452 using primarily hydrophobic interactions, with a buried surface area of $\sim 120 \text{ \AA}^2$ (Figure S3). BamD also interacts with POTRA2 along TPR1 and 2 (Figures 3B, D). While this region is less ordered in our structure, there is clearly the potential for additional electrostatic interactions here in which BamD could also participate in modulating the conformation of the POTRA domains of BamA during OMP biogenesis.

While it has been well documented that BamD interacts with BamA, it has not been shown that BamE also interacts with BamA, although the association of BamE with BamD has been shown to enhance interaction with BamA (9, 12). In our crystal structure, we observe not only the interaction of BamE with BamD, but also an extensive interaction of BamE with POTRA5 of BamA through a buried surface area of $\sim 750 \text{ \AA}^2$ (Figures 3B, E and Table S6). This binding interface is composed of numerous hydrogen bonds via side chain and backbone atoms, as well as hydrophobic interactions primarily from W376 of BamA which constitutes $\sim 130 \text{ \AA}^2$ of buried surface area alone. Residue Y28 of BamE also interacts with periplasmic loop 3 of BamA forming hydrogen bonds with E521 and P518. These interactions anchor the N-terminus of BamE in close proximity to the barrel domain of BamA, which may serve to orient BamCDE optimally for interacting with BamA (Figures 3A, F). The observation that BamE bridges an additional contact between BamD and BamA agrees well with previous work indicating that the role of BamE may be to enhance the interaction of BamD with BamA (12). Previous studies using NMR identified BamE residues that were affected by BamD binding (23); however, not all these residues mapped to the interface observed in our crystal structure. Instead, residues Y37, L38, L63, and T61 lie along the interface which interacts with POTRA5 of BamA, suggesting that binding of BamD may lead to conformational changes in BamE that promote association with BamA. BamE has also been shown to bind phosphatidylglycerol (PG), suggesting that this may contribute to its role within the BAM complex (23). The BamE residues that were identified to interact most extensively with PG (G60, T70, N71, V76, and F95) were found sitting within the periplasm as far as $35 \text{ \AA}$ from the OM. Therefore, it is unlikely that the PG-binding role of BamE contributes directly to the role of the BAM complex; however, it is

still possible that the recruitment of PG into proximity of the BAM complex could increase the efficiency of OMP biogenesis.

No structure of full-length BamA from *E. coli* has been reported previously, although a structure containing the β -barrel domain and POTRA5 is available (16). In this structure, POTRA5 was oriented away from the barrel domain in an open conformation allowing direct access to the barrel lumen from the periplasm (Figure S4). However, in our structure, which contains full-length BamA, POTRA5 was found in a closed conformation relative to the β -barrel domain, where it fully occludes access to the barrel lumen from the periplasm. Compared to the full-length BamA structure from *N. gonorrhoeae* (18), the POTRA domains undergo $\sim 90^\circ$ rotation along the plane of the membrane upon interaction with BamCDE. POTRA5 makes numerous contacts with periplasmic loops 1, 2, 3, 4, and 6, with the most extensive interactions through periplasmic loops 1, 2 and 4 (Figure S5). This conformation constitutes $\sim 45^\circ$ hinge-like conformational change of POTRA5 to the closed state (Figure 4A). POTRA5 interacts most extensively with periplasmic loop 4, which sits tucked inside the barrel domain (Figure S5 and Movie S2). Here, two important interactions stabilize the conformation observed in our crystal structure. These include a salt bridge between E396 (POTRA5) and R583 (periplasmic loop 4) and π stacking between R421 (POTRA5) and Y585 (periplasmic loop 4). Conformational flexibility of the POTRA domains has been observed for many years (28, 31, 32), so it remains to be determined whether the association of BamCDE is solely responsible for the closed conformation observed in our crystal structure.

A surprising finding of this work is that binding of BamCDE leads to an unprecedented shift of the β -barrel domain of BamA, where strands $\beta 1 - \beta 8$ undergo a conformational twist with the most dramatic change emanating from strand $\beta 1$ ($\sim 45^\circ$) and gradually diminishing until strand $\beta 9$. This conformation is likely due to the strong interactions of POTRA5 with BamCDE and with periplasmic loop 4, which impose mechanical strain on the first half of the barrel strands (Figures 4B, D, E and Movie S2). This in effect, leads to a dramatic change in the angle of the strands in the membrane such that strands $\beta 1$ and $\beta 16$ no longer associate as a β -sheet, thereby leading to opening of the exit pore along extracellular loops 1, 2 and 3. Here, the exit pore is fully open with dimensions of $\sim 15 \text{ \AA} \times \sim 27 \text{ \AA}$. This agrees with recent studies that have shown that disulfide crosslinking the exit pore in a closed state renders the BAM complex nonfunctional (24). Aligning BamA from our complex structure with the previously reported BamA structure containing POTRA5 only (PDB ID 4C4V), we calculated an RMSD of $1.07 \text{ \AA}$ for the entire β -barrel domain. However, the calculated RMSD of strands $\beta 1 - \beta 8$ alone was $2.74 \text{ \AA}$, while the RMSD for strands $\beta 9 - \beta 16$ was $0.67 \text{ \AA}$. These differences highlight the drastic shift observed in our structure.

While the conformational change of the β -barrel domain led to opening of extracellular loops 1, 2, and 3 along the exit pore, the remaining loops 4, 5, 6, 7, and 8 remained largely unchanged. The exception is that loop 4 undergoes a slight shift to stabilize loop 6, such that loop 6 is now entirely visible in our crystal structure, including the disulfide bond formed by residues C690 and C700 (Figures 4C, D). The rest of loop 6, including the conserved VRGF motif, was unchanged in conformation (Figure S6). Another consequence of the conformational twist of strands $\beta 1 - \beta 8$ is structural rearrangement at the lateral gate such that

strand $\beta 1$ no longer interacts with strand $\beta 16$ (last strand) in barrel closure, in contrast to what has been observed in all other OMPs with known structure (Figures 4F, G). Rather, most of strand $\beta 16$ sits tucked inside the barrel domain, with the C-terminus stabilized by R632, while periplasmic loop 7 and strand $\beta 15$ contact strand $\beta 1$ at an offset angle of $\sim 45^\circ$ (Figures 4D, E, F, G). This is in agreement with recent studies that rendered the BAM complex non-functional by using disulfide crosslinking to prevent such conformational changes at the lateral gate (24).

Based on our crystal structure of BamACDE and the existing crystal structure of BamAB (14), here we report the structure of the fully assembled BAM complex from *E. coli* (Movies S1, S2, and Model S1). As further validation, our structure is in agreement with a model for the BAM complex that we recently reported which was based on all structural, functional, genetics, and biochemical studies to date (33). The exceptions are that the model contained an open conformation of the periplasmic domain of BamA and no conformational changes were predicted in the β -barrel domain. Our structure reveals that upon binding BamCDE, the β -barrel domain of BamA undergoes an unprecedented conformational twist that dramatically changes the angle of the strands (shear) in the membrane, leading to opening of the exit pore and rearrangement at the lateral gate. This suggests that binding of BamCDE modulates the conformation of BamA, possibly serving as a regulatory step to increase the efficiency of OMP biogenesis by the BAM complex. The conformational changes in the β -barrel domain of BamA may serve a number of functions. For example, a longstanding question is how the BAM complex is able to fold and insert OMPs with diverse properties such as differing strand and shear numbers. It is possible that association of BamCDE may serve to ‘tune’ the β -barrel domain of BamA to fold OMPs with differing shear numbers by adjusting the angle of the strands in the membrane to match that of the substrate OMPs. This would be especially important if newly forming OMPs indeed utilize the exposed strands of BamA for strand templating by β -augmentation which has been suggested previously. Another possible function of the conformational changes observed in the β -barrel domain of BamA may be to further destabilize or even part the local membrane, thereby reducing the kinetic barrier for OMP insertion (34).

It has been suggested that substrate OMPs may be threaded through the barrel domain of BamA during biogenesis. However, in our crystal structure of the BamACDE complex, access to the interior of the barrel is completely occluded from the periplasm. Therefore, OMPs are likely inserted into the membrane at the lateral gate of BamA rather than first being threaded through the barrel domain. The lateral gate is positioned central to the BAM complex and would be directly accessible for substrate handoff by the accessory lipoproteins and/or by the POTRA domains of BamA.

Materials and Methods

Expression of recombinant BamABCDE complex

A single plasmid (pJH114) containing all five Bam proteins (BamA, B, C, D, and E) was obtained from Harris Bernstein and used for expression and purification (11). The plasmid was transformed into BL21(DE3) cells (NEB), plated onto LB-carbenicillin agar plates (Teknova) and incubated overnight at 37°C . A single colony was used to inoculate a 5-mL

LB-ampicillin culture and incubated overnight at 37°C. The overnight culture was then used to inoculate a 50 mL starter culture of LB-ampicillin which was allowed to grow to saturation. The cells were then centrifuged, washed three times with 1x PBS and then re-suspended in 12 mL 1x PBS. The resuspended cells (1 mL) were then added to twelve 2 L baffled flasks containing 1 L of 2xYT medium supplemented with ampicillin (50 µg/ml). These cultures were incubated at 37°C with shaking at 180 rpm, grown to an OD₆₀₀ between 0.8–1.0, and then induced with 0.5 mM IPTG. Cultures were grown an additional 4 hours at 37°C before harvesting. Cells were either used immediately or flash frozen and stored at –20°C.

Purification and crystallization

Cells were resuspended in lysis buffer (1x PBS supplemented with DNase I (10 µg/ml) and PMSF (500 µM)) and lysed with three passes through an Emusiflex C-3 high pressure homogenizer (Avestin) at 18,000 psi. The cell lysate was then centrifuged at 6000 x *g* for 10 min at 4°C to remove cell debris, and the resulting supernatant was centrifuged at 200,000 x *g* for 90 min at 4°C to isolate cell membranes. The membranes were then re-suspended in solubilization buffer (1x PBS, 1% DDM, and 37 mM imidazole) using a dounce homogenizer and stirred at medium speed overnight at 4°C. The solubilized sample was then centrifuged again at 200,000 x *g* for 60 min at 4°C and the supernatant collected.

Solubilized BamABCDE complex was purified by affinity chromatography using a 5 mL HiTrap Nickel column (Qiagen) and an ÄKTA Pure system (GE Healthcare). The column was equilibrated with Buffer A (1x PBS, 0.03% DDM, and 37 mM imidazole) and the sample automatically loaded using the sample pump with an in-line air sensor. Protein was eluted with a linear gradient of 37–500 mM imidazole using Buffer A and Buffer B (1x PBS, 0.03% DDM, and 1 M imidazole). Fractions containing BamABCDE were pooled, concentrated to ~2 mg/mL, and passed through a 16/60 Sephacryl S-300 HR column (GE Healthcare) at a flow rate of 0.5 mL/min using 25 mM Tris-HCl, pH 7.5, 150 mM NaCl, and 0.6% C₈E₄. All five Bam proteins (BamA, B, C, D, and E) eluted from the gel filtration column as a single monodisperse peak as verified by SDS-PAGE analysis. Fractions containing BamABCDE were pooled and concentrated to ~12 mg/mL.

Broad crystallization screening was performed using hanging drop method on a Mosquito LCP crystallization robot (TTP Labtech) with commercially available crystallization screens. An initial hit was improved by additive screening using the AdditiveHT screen (Hampton Research) with final crystals grown at 22°C in 100 mM Tris-HCl, pH 8.5, 200 mM MgCl₂, 10 mM MnCl₂, and 8% PEG 4000.

Data collection, structure determination, and modeling

Crystals were harvested by quick transfer directly into a cryoprotectant solution containing 20% glycerol and flash-cooled in liquid nitrogen. Diffraction data were collected to 3.4 Å resolution at the SER-CAT beamline (ID22) at the Advanced Photon Source at Argonne National Laboratory and processed using HKL2000 (35). The structure was solved by molecular replacement using Phaser (36) within PHENIX (37) using previously reported crystal structures of the Bam components. Search order was key here for success, first

starting with the BamCD complex (PDB ID 3TGO) followed by the barrel domain of BamA (PDB ID 4C4V), POTRA5 and then POTRA4 of BamA (PDB ID 3Q6B). BamE (PDB ID 2KM7) and POTRA1 (PDB ID 3EFC) were then placed based on density within a difference ($F_o - F_c$) map. After several rounds of building and refinement, POTRA2 and 3 (PDB ID 3EFC) were then manually placed in weak density followed by rigid body refinement for all components for final placement. The structure was refined to R/R_{free} values of 0.22/0.27. All model building and refinement were performed using COOT (38) and PHENIX (37), respectively. Final placement of side chains was based on evaluation of $2F_o - F_c$, $F_o - F_c$, and feature-enhanced (FEM) density maps (39). RMSD analysis was performed within PyMOL (Schrödinger) for C- α atoms using default settings. Surprisingly, BamB was not found within our crystal structure despite it being present in our purification. The absence of BamB was confirmed by analyzing crystals and our initial sample of the complex by SDS-PAGE analysis, which also were lacking BamB presumably due to proteolysis during storage/incubation.

To see how BamB interacts with the BamACDE complex, we modeled BamB into our complex using the previous reported BamAB crystal structure (PDB ID 4PK1) to produce the modeled structure of the fully assembled BamABCDE complex. Here, we were able to place BamB in our structure by performing a superposition of the two structures along POTRA3 of BamA. Analysis of interacting interfaces was performed using the PDBePISA (40). All figures were made with PyMOL (Schrödinger) and annotated and finalized with Adobe Illustrator.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank Harris Bernstein for providing the pJH114 plasmid. JB and NN are supported by the Department of Biological Sciences at Purdue University and by the National Institute of Allergy and Infectious Diseases (1K22AI113078-01). SKB is supported by the Intramural Research Program of the NIH, National Institute of Diabetes and Digestive and Kidney Diseases. We thank the staff at the Southeast Regional Collaborative Access Team (SER-CAT) beamline at the Advanced Photon Source, Argonne National Laboratory for their assistance during data collection. Use of the Advanced Photon Source was supported by the US Department of Energy, Office of Science, Office of Basic Energy Sciences, under Contract No. W-31-109-Eng-38 (SER-CAT).

References

1. Webb CT, Heinz E, Lithgow T. Evolution of the beta-barrel assembly machinery. *Trends Microbiol.* 2012; 20:612–620. [PubMed: 22959613]
2. Schleiff E, Soll J. Membrane protein insertion: mixing eukaryotic and prokaryotic concepts. *EMBO Rep.* 2005; 6:1023–1027. [PubMed: 16264426]
3. Knowles TJ, Scott-Tucker A, Overduin M, Henderson IR. Membrane protein architects: the role of the BAM complex in outer membrane protein assembly. *Nat Rev Microbiol.* 2009; 7:206–214. [PubMed: 19182809]
4. Hagan CL, Silhavy TJ, Kahne D. beta-Barrel membrane protein assembly by the Bam complex. *Annu Rev Biochem.* 2011; 80:189–210. [PubMed: 21370981]
5. Voulhoux R, Bos MP, Geurtsen J, Mols M, Tommassen J. Role of a Highly Conserved Bacterial Protein in Outer Membrane Protein Assembly. *Science.* 2003; 299:262–265. [PubMed: 12522254]

6. Noinaj N, Rollauer SE, Buchanan SK. The beta-barrel membrane protein insertase machinery from Gram-negative bacteria. *Curr Opin Struct Biol*. 2015; 31:35–42. [PubMed: 25796031]
7. Kim KH, Aulakh S, Paetzel M. The bacterial outer membrane beta-barrel assembly machinery. *Protein Sci*. 2012; 21:751–768. [PubMed: 22549918]
8. Ricci DP, Silhavy TJ. The Bam machine: A molecular cooper. *Biochim Biophys Acta*. 2012; 1818:1067–1084. [PubMed: 21893027]
9. Malinverni JC, Werner J, Kim S, Sklar JG, Kahne D, Misra R, Silhavy TJ. YfiO stabilizes the YaeT complex and is essential for outer membrane protein assembly in *Escherichia coli*. *Mol Microbiol*. 2006; 61:151–164. [PubMed: 16824102]
10. Hagan CL, Kim S, Kahne D. Reconstitution of Outer Membrane Protein Assembly from Purified Components. *Science*. 2010; 328:890–892. [PubMed: 20378773]
11. Roman-Hernandez G, Peterson JH, Bernstein HD. Reconstitution of bacterial autotransporter assembly using purified components. *Elife*. 2014; 3:e04234. [PubMed: 25182416]
12. Sklar JG, Wu T, Gronenberg LS, Malinverni JC, Kahne D, Silhavy TJ. Lipoprotein SmpA is a component of the YaeT complex that assembles outer membrane proteins in *Escherichia coli*. *Proceedings of the National Academy of Sciences*. 2007; 104:6400–6405.
13. Kim KH, Aulakh S, Paetzel M. Crystal structure of beta-barrel assembly machinery BamCD protein complex. *J Biol Chem*. 2011; 286:39116–39121. [PubMed: 21937441]
14. Jansen KB, Baker SL, Sousa MC. Crystal structure of BamB bound to a periplasmic domain fragment of BamA, the central component of the beta-barrel assembly machine. *J Biol Chem*. 2015; 290:2126–2136. [PubMed: 25468906]
15. Albrecht R, Zeth K. Structural basis of outer membrane protein biogenesis in bacteria. *J Biol Chem*. 2011; 286:27792–27803. [PubMed: 21586578]
16. Albrecht R, Schutz M, Oberhettinger P, Faulstich M, Bermejo I, Rudel T, Diederichs K, Zeth K. Structure of BamA, an essential factor in outer membrane protein biogenesis. *Acta Crystallographica Section D*. 2014; 70:1779–1789.
17. Noinaj N, Fairman JW, Buchanan SK. The Crystal Structure of BamB Suggests Interactions with BamA and Its Role within the BAM Complex. *J Mol Biol*. 2011; 407:248–260. [PubMed: 21277859]
18. Noinaj N, Kuszak AJ, Gumbart JC, Lukacik P, Chang H, Easley NC, Lithgow T, Buchanan SK. Structural insight into the biogenesis of beta-barrel membrane proteins. *Nature*. 2013; 501:385–390. [PubMed: 23995689]
19. Heuck A, Schleiffer A, Clausen T. Augmenting beta-Augmentation: Structural Basis of How BamB Binds BamA and May Support Folding of Outer Membrane Proteins. *J Mol Biol*. 2011; 406:659–666. [PubMed: 21236263]
20. Kim KH, Paetzel M. Crystal Structure of *Escherichia coli* BamB, a Lipoprotein Component of the beta-Barrel Assembly Machinery Complex. *J Mol Biol*. 2011; 406:667–678. [PubMed: 21168416]
21. Dong C, Hou HF, Yang X, Shen YQ, Dong YH. Structure of *Escherichia coli* BamD and its functional implications in outer membrane protein assembly. *Acta Crystallogr D Biol Crystallogr*. 2012; 68:95–101. [PubMed: 22281737]
22. Ni D, Wang Y, Yang X, Zhou H, Hou X, Cao B, Lu Z, Zhao X, Yang K, Huang Y. Structural and functional analysis of the β -barrel domain of BamA from *Escherichia coli*. *The FASEB Journal*. 2014; 28:2677–2685. [PubMed: 24619089]
23. Knowles TJ, Browning DF, Jeeves M, Maderbocus R, Rajesh S, Sridhar P, Manoli E, Emery D, Sommer U, Spencer A, Leyton DL, Squire D, Chaudhuri RR, Viant MR, Cunningham AF, Henderson IR, Overduin M. Structure and function of BamE within the outer membrane and the beta-barrel assembly machine. *EMBO Rep*. 2011; 12:123–128. [PubMed: 21212804]
24. Noinaj N, Kuszak Adam J, Balusek C, Gumbart James C, Buchanan Susan K. Lateral Opening and Exit Pore Formation Are Required for BamA Function. *Structure*. 2014; 22:1055–1062. [PubMed: 24980798]
25. Jansen KB, Baker SL, Sousa MC. Crystal structure of BamB from *Pseudomonas aeruginosa* and functional evaluation of its conserved structural features. *PLoS One*. 2012; 7:e49749. [PubMed: 23189157]

26. Webb CT, Selkig J, Perry AJ, Noinaj N, Buchanan SK, Lithgow T. Dynamic association of BAM complex modules includes surface exposure of the lipoprotein BamC. *J Mol Biol.* 2012; 422:545–555. [PubMed: 22683355]
27. Sandoval CM, Baker SL, Jansen K, Metzner SI, Sousa MC. Crystal structure of BamD: an essential component of the beta-Barrel assembly machinery of gram-negative bacteria. *J Mol Biol.* 2011; 409:348–357. [PubMed: 21463635]
28. Kim S, Malinverni JC, Sliz P, Silhavy TJ, Harrison SC, Kahne D. Structure and Function of an Essential Component of the Outer Membrane Protein Assembly Machine. *Science.* 2007; 317:961–964. [PubMed: 17702946]
29. Ricci DP, Hagan CL, Kahne D, Silhavy TJ. Activation of the Escherichia coli beta-barrel assembly machine (Bam) is required for essential components to interact properly with substrate. *Proc Natl Acad Sci U S A.* 2012; 109:3487–3491. [PubMed: 22331884]
30. Rigel NW, Ricci DP, Silhavy TJ. Conformation-specific labeling of BamA and suppressor analysis suggest a cyclic mechanism for beta-barrel assembly in Escherichia coli. *Proc Natl Acad Sci U S A.* 2013; 110:5151–5156. [PubMed: 23479609]
31. Gatzeva-Topalova PZ, Walton TA, Sousa MC. Crystal Structure of YaeT: Conformational Flexibility and Substrate Recognition. *Structure.* 2008; 16:1873–1881. [PubMed: 19081063]
32. Gatzeva-Topalova PZ, Warner LR, Pardi A, Sousa MC. Structure and flexibility of the complete periplasmic domain of BamA: the protein insertion machine of the outer membrane. *Structure.* 2010; 18:1492–1501. [PubMed: 21070948]
33. O’Neil PK, Rollauer SE, Noinaj N, Buchanan SK. Fitting the Pieces of the beta-Barrel Assembly Machinery Complex. *Biochemistry.* 2015; 54:6303–6311. [PubMed: 26394220]
34. Gessmann D, Chung YH, Danoff EJ, Plummer AM, Sandlin CW, Zaccari NR, Fleming KG. Outer membrane beta-barrel protein folding is physically controlled by periplasmic lipid head groups and BamA. *Proc Natl Acad Sci U S A.* 2014; 111:5878–5883. [PubMed: 24715731]
35. Otwinowski, Z.; Minor, W. *Methods Enzymol.* Vol. 276. Academic Press; New York: 1997. p. 307–326.
36. McCoy AJ, Grosse-Kunstleve RW, Adams PD, Winn MD, Storoni LC, Read RJ. Phaser crystallographic software. *J Appl Crystallogr.* 2007; 40:658–674. [PubMed: 19461840]
37. Adams PD, Afonine PV, Bunkoczi G, Chen VB, Davis IW, Echols N, Headd JJ, Hung LW, Kapral GJ, Grosse-Kunstleve RW, McCoy AJ, Moriarty NW, Oeffner R, Read RJ, Richardson DC, Richardson JS, Terwilliger TC, Zwart PH. PHENIX: a comprehensive Python-based system for macromolecular structure solution. *Acta Crystallogr D Biol Crystallogr.* 2010; 66:213–221. [PubMed: 20124702]
38. Emsley P, Lohkamp B, Scott WG, Cowtan K. Features and development of Coot. *Acta Crystallogr D Biol Crystallogr.* 2010; 66:486–501. [PubMed: 20383002]
39. Afonine PV, Moriarty NW, Mustyakimov M, Sobolev OV, Terwilliger TC, Turk D, Urzhumtsev A, Adams PD. FEM: feature-enhanced map. *Acta Crystallogr D Biol Crystallogr.* 2015; 71:646–666. [PubMed: 25760612]
40. Krissinel E, Henrick K. Inference of macromolecular assemblies from crystalline state. *J Mol Biol.* 2007; 372:774–797. [PubMed: 17681537]

Fig. 1. The structure of the BAM complex

A. The pathway for the biogenesis of β -barrel outer membrane proteins in Gram-negative bacteria. **B.** A membrane view of the structure of the full BAM complex, formed from merging the crystal structures of BamACDE and BamAB (PDB ID 4PK1). The bottom panel shows the periplasmic view, rotated 90° along the x -axis relative to the top panel. BamA is shown in green, BamB in gray, BamC in blue, BamD in gold, and BamE in purple. **C.** The β -barrel domain of BamA undergoes a dramatic conformational change along strand β 1- β 8, which can be seen here. This panel is rotated $\sim 90^\circ$ along the y -axis relative to the top view of panel B. The overall measurements of the BAM complex are $\sim 115 \times 115 \times 115$ Å.

Fig. 2. Interactions of BamC and BamE with BamD

A. The complex structure of BamCDE is shown here with BamD in gold, BamC in blue, and BamE in purple. The TPR domains of BamD are indicated. The bottom panel is rotated 90° along the x-axis relative to the top. **B.** The interactions between BamC and BamD are nearly the same as that observed in the previously reported crystal structure (PDB ID 3TGO) with an RMSD of 1.25 Å across both chains. The bottom panel is rotated 90° along the x-axis relative to the top. **C.** View showing the interactions of BamE with BamD TPR4 and 5 through an extensive interface with a buried surface area of ~800 Å², containing a mix of hydrogen bonds, salt bridges, and hydrophobic interactions. **D.** A view from the top which is rotated 90° along the x-axis to further illustrate the extensive binding interface.

Fig. 3. Interactions of BamD and BamE with BamA

A. BamD interacts primarily along POTRA5 of BamA via TPR4 and 5, but also along POTRA2 via TPR1 and 2, while BamE interacts along POTRA5. **B.** A rotated view without the β -barrel domain of BamA highlighting the interacting regions. **C.** View of the interaction of BamD with POTRA5 of BamA, showing an extensive binding interface with a buried surface area of $\sim 1,100 \text{ \AA}^2$, containing a mix of hydrogen bonds, salt bridges, and hydrophobic interactions. Residue R197 of BamD clearly forms a salt bridge with E373 of BamA. Residue D481 (green box) is from periplasmic loop 2 of BamA. **D.** View of the interactions between TPR1 and 2 of BamD with POTRA2 of BamA. While minimal here, these interactions could assist in modulating the conformation of BamA. **E.** View of the interactions of BamE with POTRA5 of BamA, an extensive interaction with a buried surface area of $\sim 750 \text{ \AA}^2$, containing a mix of hydrogen bonds, salt bridges, and hydrophobic interactions. **F.** The N-terminal region of BamE was found anchored via hydrogen bonding interactions of residue Y28 to residues E521 and P518 of BamA periplasmic loop 3.

Fig. 4. Conformational changes in the barrel domain of BamA

A. Superposition of BamA from our structure (green) with PDB ID 4C4V (cyan) reveals $\sim 45^\circ$ hinge-like conformational change of the POTRA domains to a closed, periplasm occluded state. **B.** Binding of BamCDE to BamA leads to an unprecedented twist of strands $\beta 1$ - $\beta 8$ with the most dramatic change emanating from strand $\beta 1$ ($\sim 45^\circ$) and gradually diminishing until strand $\beta 9$ (Movie S2). This leads to opening of the exit pore and lateral gate. **C.** In response to the shift of the barrel strands, an opening of surface loops 1, 2, and 3 was observed; however, surface loops 4, 5, 6, 7, and 8 were mostly unchanged. **D.** Rotated view highlighting the strand shift along strands $\beta 1$ - $\beta 8$ of the barrel domain of BamA. **E.** View of the strand shift along strands $\beta 1$ - $\beta 8$ rotated $\sim 90^\circ$ relative to panel D illustrating a twist of the strands rather than just a simple rotation. **F.** View of the lateral gate in BamA where strand $\beta 1$ and $\beta 16$ no longer form a β -sheet interaction to close the barrel, rather, strands $\beta 15$ and $\beta 16$ are situated at $\sim 45^\circ$ angle relative to strand $\beta 1$. **G.** View showing residues along the lateral gate with $\beta 16$ sitting tucked inside the barrel and periplasmic loop 7 interacting with strand $\beta 1$.